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SEROLOGICAL STUDIES OF ATLANTIC REDFISH

BY CARL J. SINDERMANN

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ABSTRACT

Individual variations in erythrocyte antigens have been found in redfish, *Sebastes marinus*, from the western North Atlantic. Two closely related antigens, tentatively labeled A_1 and A_2, were demonstrated with specific reagents created by absorptions of rabbit antisera. Each reagent would agglutinate cells of only one antigenic type, so it was possible to identify fish as possessing A_1 or A_2 antigen. Individuals with the erythrocyte antigen A_1 make up more than 75 percent of the Eastport (Maine) redfish population. Since antigen frequencies may vary from one population to another, quantitative studies of each major fishing area should provide further information about discreteness of groups and the extent of their movements.

SEROLOGICAL STUDIES OF ATLANTIC REDFISH

By Carl J. Sindermann, *Fishery Research Biologist*
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Knowledge of the population structure of commercial marine fish species is important as a basis for management. Traditionally, information about intraspecies groups has been derived from morphometric and meristic studies, tagging, or age and growth studies. Valuable data have been obtained through the use of such methods, and it seems reasonable that the greater the number of criteria used, the more accurate will be the conclusions. For this reason, and because of the ultimate need for genetic information about intraspecies groups, attention has been directed recently toward serological methods of population analysis, and particularly toward blood-group research. Serological studies of marine fishes (Cushing, 1952, 1956; Ridgway, 1957; Ridgway, Cushing, and Durall, 1958; Suzuki, Shimizu, and Morio, 1958; Sindermann and Mairs, 1959; Ridgway and Klontz, 1960) have demonstrated individual differences in red-blood-cell antigens, and, in some studies quantitative differences in the frequency of occurence of antigens in different populations. Knowledge of such differences should prove as useful in fishery investigations concerned with population or racial problems as that of the racial distribution of human blood groups has to anthropological studies.

Before quantitative studies are possible, however, the often slow but always essential development of blood-group systems and standardization of reagents for their demonstration must be carried out. This paper is concerned with the recognition and description of blood-group antigens in redfish, *Sebastes marinus* (L.), which in recent years has become a commercially valuable species in the western North Atlantic. Attention has been directed to this fish because of confusion about certain morphologically recognizable groups which are possibly of subspecies stature, and because of a lack of knowledge concerning the population structure within these groups. Such problems should be amenable to serological investigation; the blood group antigens described here represent a first, tentative, but necessary step in this process.

METHODS

Samples of redfish blood were obtained from Eastport, Maine, and Georges Bank in the southern Gulf of Maine. Blood was taken directly from the heart; cells for testing were washed from clots with a 1.4 percent saline solution and were used in approximately 4 percent suspensions. Antisera were prepared in rabbits and chickens by injections of individual and pooled samples of washed redfish blood cells diluted 1:1 with saline. Usually, a single, short injection series was used to develop as specific antisera as possible. Six injections of 1 milliliter each were given on alternate days. Rabbits were trial bled 10 days after the last injection, and, if the antiserum titer proved satisfactory, food was withheld and the animals were bled terminally on the following day. Rabbit antisera were also prepared by injecting red cells of cod, herring, alewife, lamprey, and sheep.

Cell agglutination tests were made at room temperature within 72 hours from the time the fish blood was taken, although reactions of refrigerated cells did not change noticeably up to 7 days. Tube agglutination tests used 0.2 ml. antiserum dilution and 0.05 ml. cell suspension. Readings were taken after 15 minutes incubation at room

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temperature and 30 seconds centrifugation. In the preparation of specific agglutinating reagents, antisera were absorbed by incubating one part redfish cells and four parts diluted antiserum for 10 minutes at room temperature. One absorption was usually sufficient to remove all antibodies reactive with the absorbing cells. Reagents and antisera were frozen in 3-ml. aliquots which were thawed and heat-inactivated just before use to destroy complement.

RESULTS

Rabbit antisera prepared by injecting pooled and individual samples of redfish cells provided the first indication of individual differences in redfish erythrocytes. At appropriate dilutions of these antisera, cells of certain fish reacted weakly, while cells of other fish were strongly positive. As an example, a rabbit antiserum (labeled GBR17R), prepared by injecting pooled cells of 14 Georges Bank redfish, agglutinated cells of certain redfish from a small sample taken at Eastport, Maine, to a titer of 1:128, while cells from other fish in the same sample were not agglutinated beyond the 1:32 dilution (table 1). The degree of agglutination was recorded conventionally in descending order from (+±++), representing complete agglutination, to (−), indicating no agglutination.

TABLE 1.—*Reactions of rabbit anti-redfish serum* (GBR17R) *with erythrocytes of six individual Eastport redfish*

Antiserum GBR17R dilutions	Degree of agglutination of erythrocytes from individual redfish					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
1:8	++	++++	++++	++	++++	++++
1:16	++	+++	+++	++	++++	+++
1:32	+	++	++	+	+++	++
1:64	−	+	+	−	++	+
1:128	−	+	−	−	+	+

Cells from fish Nos. 1 and 4 reacted weakly with the antiserum, while cells of other fish reacted more strongly. This suggestion of individual differences in redfish erythrocyte antigens was supported by absorptions of the antiserum. Results of absorbing with cells from each of the fish mentioned above are presented in table 2.

Absorption of the antiserum with cells of fish Nos. 2, 3, 5, and 6 removed antibodies for absorbing cells and for all others, while absorption with

TABLE 2.—*Results of absorbing*[1] *rabbit anti-redfish seru* (GBR17R) *with erythrocytes of six individual Eastpo redfish*

Antiserum (GBR17R) diluted 1:4 and absorbed with erythrocytes of individual redfish	Degree of agglutination of erythrocytes from individu redfish					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
No. 1	−	+	++	−	+	+
No. 2	−	−	−	−	−	−
No. 3	−	−	−	−	−	−
No. 4	−	+	+	−	+	+
No. 5	−	−	−	−	−	−
No. 6	−	−	−	−	−	−
Unabsorbed	++	++++	++++	++	++++	+++

[1] Absorptions of specific antibodies were made by incubating one pa redfish cells and 4 parts diluted (1:4) antiserum for 10 minutes at room te perature. After this period, the absorbing cells were centrifuged and t supernatant fluid tested against an aliquot of the cells used for absorptic One absorption was usually sufficient to remove all antibodies reactive wi antigens of the absorbing cells.

cells of fish Nos. 1 and 4, which reacted weakl with unabsorbed antiserum, removed antibodie for each other, but left antibodies which agglut nated cells of fish Nos. 2, 3, 5, and 6. Compariso of cell agglutinations with absorbed and unal sorbed antiserum indicated that absorptions drast cally reduced the antibody content. The tes demonstrated antigenic dissimilarity in redfis erythrocytes, but suggested that the antigens wer closely related. As a descriptive measure, fis whose cells reacted strongly with unabsorbed ant serum, and also removed antibodies for all othe cells in absorptions, were considered as possessin an antigen A_1, while those fish whose cells reacte weakly with the unabsorbed antiserum, and di not remove antibodies for A_1 cells in absorption were considered as possessing an antigen A_2.

Referring again to table 2, weakly reacting cel of fish Nos. 1 and 4 would thus be A_2, whil strongly reacting cells of fish Nos. 2, 3, 5, and would be A_1. Using a reagent prepared by ab sorbing the GBR17R antiserum with cells of A fish, it was possible to recognize fish of each anti genic type, since only cells of fish with A_1 antige were agglutinated.

Confirmation of this system was possible with rabbit antiserum (labeled MIC8R) which had bee prepared for another study by injecting poole cod cells. Individual differences were slight wit the unabsorbed antiserum, but absorption wit cells of the six fish used previously produced re sults shown in table 3.

Absorption with cells of fish Nos. 1 and 4 re moved antibodies for absorbing cells and for al

TABLE 3.—*Results of absorbing rabbit anti-cod serum* (MIC8R) *with erythrocytes of six individual Eastport redfish*

Antiserum IC8R diluted 1:4 and absorbed with rythrocytes of ndividual redfish	Degree of agglutination of erythrocytes from individual redfish					
	1 (A_2)	2 (A_1)	3 (A_1)	4 (A_2)	5 (A_1)	6 (A_1)
o. 1	−	−	−	−	−	−
o. 2	+	−	−	+	−	−
o. 3	+	−	−	+	−	−
o. 4	−	−	−	−	−	−
o. 5	+	−	−	+	−	−
o. 6	+	−	−	+	−	−
nabsorbed	++	+++	+++	++	++++	+++

thers, while absorption with cells of fish Nos. 2, , 5, and 6 removed antibodies for each other but ft antibodies which agglutinated cells of fish os. 1 and 4. These reactions were reciprocals of hose obtained by absorbing the anti-redfish GBR17R) serum. They immediately suggested hat the anti-cod (MIC8R) was principally an nti-A_2 serum, while the anti-redfish (GBR17R) vas principally an anti-A_1 serum. Therefore, by bsorbing the MIC8R antiserum with A_1 cells No. 2, 3, 5, and 6, and others with similar reaction), a reagent was obtained which reacted nly with A_2 cells. This provided two reagents, ne (prepared previously by absorbing GBR17R ntiserum with A_2 cells) which agglutinated only ells with A_1 antigen; the other (prepared by absorbing MIC8R antiserum with A_1 cells) which gglutinated only cells with A_2 antigen. It was hen possible to definitely identify fish as possessng either antigen A_1 or A_2.

Since the original stock of MIC8R antiserum vas small, other specific anti-redfish sera were prepared. Rabbits were immunized with cells of ndividual redfish previously tested for A antigens. Two antisera (GBR31R and GBR32R) were especially useful in preparation of specific A_1 reagents. Results of absorptions of GBR31R antiserum are illustrated in table 4. More than 75 percent of redfish sampled thus far from the Eastport (Maine) population possessed antigen A_1. Future work logically involves testing large numbers of individuals from each major fishing area to determine whether variations in antigen frequencies exist. If such variations occur, the maximum amount of intermixing could be determined.

DISCUSSION

Blood-group antigens of the kind considered in this paper have been most thoroughly studied in mammals and birds. Blood-group systems have been proposed for humans, cattle, whales, chickens, and certain other animals. Wherever studied, the erythrocyte antigens composing these systems have been found to be genetically determined, with relatively simple inheritance usually involving a single locus. Comparable studies of fishes are still in their infancy, but should be as useful to studies of fish populations and migrations as human blood groups are to anthropological studies, or as blood groups of cattle are to herd-lineage problems. Information derived is genetic, and may provide a more adequate picture of the population structure of a species than is possible with morphological criteria, many of which are subject to modification by environmental factors.

From research in this laboratory (Sindermann and Mairs, 1959) and that reported from elsewhere (Cushing, 1956; Ridgway, Cushing, and Durall, 1958; Suzuki, Shimizu, and Morio, 1958; and others), it is already apparent that individual variations in erythrocyte antigens characterize

TABLE 4.—*Results of absorbing rabbit anti-redfish (anti A_1) serum* (GBR31R) *with cells of 12 Eastport (Maine) redfish*

Antiserum GBR31R absorbed with cells of redfish	Degree of agglutination of erythrocytes from individual redfish											
	61(A_1)	62(A_2)	63(A_1)	64(A_1)	65(A_1)	66(A_1)	67(A_2)	68(A_1)	69(A_2)	70(A_1)	71(A_1)	72(A_1)
No. 61	−	−	−	−	−	−	−	−	−	−	−	−
No. 62	+++	−	++++	++	+++	+++	−	+++	−	+++	++	+++
No. 63	−	−	−	−	−	−	−	−	−	−	−	−
No. 64	−	−	−	−	−	−	−	−	−	−	−	−
No. 65	−	−	−	−	−	−	−	−	−	−	−	−
No. 66	−	−	−	−	−	−	−	−	−	−	−	−
No. 67	+	−	++	+	+	+	−	++	−	+	+	+++
No. 68	−	−	−	−	−	−	−	−	−	−	−	−
No. 69	+	−	++	+	+	+	−	++	−	+	+	++
No. 70	−	−	−	−	−	−	−	−	−	−	−	−
No. 71	−	−	−	−	−	−	−	−	−	−	−	−
No. 72	−	−	−	−	−	−	−	−	−	−	−	−
Unabsorbed	++++	++	++++	++++	++++	++++	++	++++	++	++++	+++	++++

teleosts as well as other vertebrate groups. Recent findings of Suzuki, Shimizu, and Morio (1958), Sindermann and Mairs (1959), and Ridgway and Klontz (1960) suggest that geographic variation in antigen frequencies can also be expected. If this is generally true, such variations may prove important to population or racial studies of marine fishes.

The A blood-group antigens of redfish described in this paper represent a first phase in serological characterization of this important marine fish species. Future work should include quantitative studies of the distribution of A-group antigens in redfish from widely separated areas, to see whether there is variability in their frequencies. Furthermore, search should be continued for other blood-group antigens in the species, since adequate serological characterization may depend on several such criteria. Just as in humans it is possible to make major racial separations based on the ABO blood-group system alone, so an initial redfish population analysis may be based only on the A antigens. However, more precise population subdivisions and indications of past migrations of humans have emerged from studies of several blood-group systems, and more complete understanding of redfish populations could depend on identification, description, and use of more than one system. The initial serological step has been taken, with encouraging results, but more information on a much broader scale is necessary.

Use of the varied approaches available to serology, including study of serum and egg proteins as well as erythrocyte antigens comparable to those described here, should provide further data for fish systematic studies, both above and below the species level. It should be emphasized however that no single approach to problems of subspecies, races, or subpopulations can be a panacea. Serology may constitute one of several criteria in such analyses, along with traditional morphometric and meristic studies, but a final synthesis should include data from diverse methods of investigation. It does seem, though, that once techniques have been sufficiently standardized, serology will provide a powerful tool for biological studies of such commercially valuable species the redfish.

CONCLUSIONS AND SUMMARY

Individual differences in erythrocyte antige have been found to exist in redfish. Detectio of such differences was possible with absorbe rabbit anti-redfish and anti-cod serum.

Based on results of antiserum absorptions, tw closely related antigens, A_1 and A_2 have bee identified. Reagents were prepared which sp cifically agglutinated cells possessing each antige

Using the reagents prepared by absorptions antisera, it has been found in limited samplin that redfish possessing the erythrocyte antigen make up more than 75 percent of the Eastpo (Maine) population.

Quantitative studies of antigen frequencies samples from each major fishing area should pr vide information about the intraspecies structu of redfish.

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